



# NOSEMA SAMPLING

*Spore Counting Procedure to Monitor the Infestation level of Nosema Microsporidians*

Nosema is a microsporidian that can gravely affect a honeybee colony's health and performance. This disease can result in an increase in winter mortality, suppression of the immune system, and as a result will have a negative effect on colony productivity. Nosema affects the hypopharyngeal glands in honey bees causing a decrease in the production of larval food and brood rearing. Monitoring the level of Nosema in a colony provides an indication of the infestation level and is an indicator of colony health.

## Lab Analysis Supplies

1. Microscope (compound or phase)
2. 70% ethanol
3. Pasteur pipette
4. Hemocytometer with cover slip
5. Plastic bag
6. Rolling pin
7. Liquid measuring equipment (e.g., beaker)
8. Filtered water

## Field Sampling Supplies:

1. Sampling Jars
2. 70% Ethanol (or winter washer fluid)
3. Small bin/tub (e.g. dishwashing tub)
4. ¼ cup measuring cup

## STEP 1

### Choosing a Frame

Select a frame with mature bees, as they are most likely to have the highest amount of Nosema spores. These bees can be found on any frame from a honey super or an outer honey frame in the brood nest. Ensure that the queen is not present on the frame.

## STEP 2

### Collecting Bees

Shake the selected frame into the tub. Gather the bees into the corner and scoop out ¼ cup. Alternatively, you can gently scrape a ¼ cup of bees right from the frame. Pour the bees into a jar that is 1/3 full of ethanol (or washer fluid). Seal the jar tightly with the lid when finished.

## STEP 3

### Preparing the Sample

Count out 60 bees from the sample and place in a plastic bag. Macerate the bees in the bag with a rolling pin to crush into small bits (the smaller the pieces the better). Add 1 ml of filtered water per bee to the bag (in this case 60 ml) and mix by squeezing the bag.

## STEP 4

### Setting Up the Hemocytometer

Place the hemocytometer onto a flat surface with the coverslip on top. Slowly pipette a small amount of the prepared sample into each well of the hemocytometer. If some spills out, use a kim wipe or tissue to carefully soak up excess fluid. Let the sample sit for ~2 mins so the contents can settle.

## STEP 5

### Focusing the Microscope

Place the sample on the stage of the microscope. Focus on square 1 using 40X objective lens. This is the ideal magnification to see *Nosema* spores.

## STEP 6

### Recognizing a Spore

*Nosema* spores will look like rice grains and have a distinctive 'halo' around them. When counting the spores, do not count it unless you can confirm it is a spore. Do not count anything that is mis-shapen or in large clumps.



## STEP 7

### Counting the spores

Count all the spores in the 16 smaller squares within square 1. Be sure to only count the spores that land on the top and right-hand lines (in yellow). Leave out spores on the left and bottom lines to avoid double counting. Count all the spores in boxes 1-5 (see photo from step 5) and record the number after counting each box.

## STEP 8

### Calculate your Infestation Intensity

To calculate the percent infestation, you need to know the amount of liquid in each square of your hemocytometer.

Hemocytometer with a 0.2x0.2x0.1 large square size

In 1 square there is 0.000004 ml

If you counted 5 squares, the total volume is

$0.000004 \text{ ml} \times 5 = 0.00002 \text{ ml}$

Take the total number of spores counted in all 5

boxes/ 0.00002 ml = the number of spores/ml

Hemocytometer with a 0.2x0.2x0.02 large square size

In 1 square there is 0.0000008 ml

If you counted 5 squares, the total volume is

$0.0000008 \text{ ml} \times 5 = 0.000004 \text{ ml}$

Take the total number of spores counted in all 5

boxes/ 0.000004 ml = the number of spores/ml

#### Notes:

- 1ml = 1 bee, therefore the value calculated is also equal to number of spores/bee.
- The economic threshold for *Nosema* is 1 million spores per bee.
- If your hemocytometer has a different chamber size than the two listed above, a different formula will need to be used.

## STANDARD PRECAUTIONS FOR NOSEMA SAMPLING

1. BE EXTREMELY CAREFUL HANDLING ETHANOL OR WINDSHIELD WASHER FLUID AROUND A LIVE COLONY
2. MAKE SURE EACH COLONY IS SAMPLED SEPERATELY
3. MAKE SURE TO RINSE THE HEMOCYTOMETER, COVER SLIP, AND PIPETTE BETWEEN EACH SAMPLE